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Comparative antimicrobial activity of four essential oils extracted from medicinal plants harvested in South East Nigeria

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ABSTRACT:
The antimicrobial activity of the essential oils from four medicinal plants, namely, Onion, Guava, Basil and Ginger have been compared in this study using four standard strains of bacteria and one fungi. The Disc diffusion method was used to test for sensitivity, and then the Minimum Inhibitory Concentrations (MICs) determined graphically. Sensitivity tests showed all bacteria and fungi to be susceptible to all the essential oils tested. No single essential oil was observed to have a consistently higher activity than all the rest against all the tested organisms. However, Onion showed the highest activity with a very low MIC of 0.0003ml/ml against *Staphylococcus aureus,* and Ginger the lowest activity with a high MIC of 0.126ml/ml against *Pseudomonas aeruginosa.* On the other hand, *Staphylococcus aureus* is significantly ($p<0.05$), most susceptible to all the essential oils tested with MICs as low as 0.001 for Ginger, 0.001 for Basil and 0.001ml/ml for Guava. Therefore, the choice of essential oil to be used as an antimicrobial agent will depend on the micro-organism to be prevented or treated.

Key words: Essential oils, antimicrobial, disc diffusion, Minimum Inhibitory Concentration (MIC).

INTRODUCTION

The need for new antimicrobial agents has become necessary due to the problem of emergence of strains that are resistant to most antibiotics currently in use [1], [2]. Due to this fact, the medical world is currently facing a situation where some common antimicrobial agents have become less effective.

In the search for effective new antimicrobials various research works have been carried out on essential oils, investigating their antimicrobial properties. According to the report of some authors [3], who worked on five essential oils, and whose inference was based on their inhibition zone diameters, said that thyme oil had the strongest antibacterial activity against all tested bacteria except *Staphylococcus aureus* and *Escherichia coli*, with the most sensitive bacteria being *Pseudomonas aeruginosa*. Another report [4], stated that the components of essential oil of Thyme and Pine oil are highly active against food borne pathogens, generating larger inhibition zones for both Gram positive and negative bacteria. Further, studies on the comparative chemical constituents and antimicrobial activity of normal and organic ginger oils [5] indicated that organic ginger oil showed significant antimicrobial activity in comparison to normal ginger oil. Their report also indicated that although both normal and organic ginger oils contain zingiberene as their major compound, its ratio differs in the different oils.

It has been suggested that the antimicrobial activity of essential oils is a result of the antimicrobial properties of both the major and minor components in their chemical composition [6], which may enable them to destroy the cellular structure of the micro-organisms leading to death. This indicates that the bioactivity of essential oils is dependent not only on the combination of the constituents but also on the chemical structures of these compounds. The hydrophobicity of essential oils enables them to partition in the lipids of the cell membranes and mitochondria, rendering them permeable, leading to leakage of cell contents. Physical conditions that improve the action of essential oils are low pH, low temperature and low oxygen levels [7]. The same author reports that synergism has been observed between carvacrol and its precursor p-cymene, and between cinnamaldehyde and eugenol. This corresponds with a report that antimicrobial effectiveness is not only assessed through the main component, but also a synergistic effect may occur by the other components [8]. The antimicrobial efficiency of essential oils from the same plant species is often affected by harvest time, weather conditions during growth and harvest, genotype and different geographical locations where plants are grown [9].

MATERIALS AND METHODS.

Sample collection.

The fresh plant material bulbs of Onion (*Allium cepa)* Linn (Liliaceae) (UUH/038/15C); leaves of Guava (*Psidium guajava Linn (*Myrtaceae*) (UUH/036/15C);* rhizome *of* Ginger *(Zingiber officinale)* Linn (Zingiberaceae) (UUH/037/15C); leaves of African Basil (*Ocimum gratissimum)* Linn (Lamiaceae) (UUH/035/15C) were collected from the botanical garden, University of Uyo. The identities of the plants were ascertained

morphologically by Dr. (Mrs) M. E. Bassey, a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo.

Extraction.
The freshly collected plant samples were washed with distilled water, after which they were cut into pieces before extraction. The freshly cut individual samples were homogenized using a blender (50g each). Each sample was hydro-distilled for 5 hours in a Clavenger type apparatus. The oils were dried over anhydrous sodium sulphate and stored in an air tight container and then used for the antimicrobial activity studies. The percentage yields of the various oils were calculated.

Test organisms.
The microorganisms selected for this study were obtained from the Nigerian Institute of Medical Research, Lagos. They include the Gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*; Gram negative bacteria, *Pseudomonas aeruginosa* and *E. coli*; plus the fungus, *Apergillus niger*. The bacteria and fungi test organisms were maintained on freshly prepared nutrient agar and Sabouraud agar medium, respectively.

Preparation of nutrient agar
28gm of the nutrient agar powder was weighed and dissolved in a litre of sterile water in a conical flask. The solution was mixed properly and was placed in an autoclave at a temperature of 121^{o}C for thirty minutes until fully sterilized. When fully sterilized the agar was poured carefully and aseptically into the plates and allowed to solidify.

Preparation of dextrose sabouraud agar.
32.5gm of Sabouraud agar powder was weighed and dissolved in 500ml of sterilized water in a conical flask. The solution was mixed properly and placed in an autoclave at a temperature of 121^{o}C for thirty minutes until fully sterilized. When fully sterilized the agar was poured carefully and aseptically into the plates and allowed to solidify.

Preparation of nutrient broth.
6.5gm of nutrient broth powder was weighed and dissolved in 500ml of sterilized water in a conical flask. The solution was mixed properly and dispensed into final containers. Sterilization was carried out by autoclaving at a temperature of 121^{o}C for thirty minutes until fully sterilized.

Antimicrobial activity test.
For antimicrobial assay, nutrient agar plates were inoculated with 0.2mL of overnight growth culture of each test bacterial suspension. Similarly, Sabouraud agar plates were also inoculated with 0.2mL of cultured test fungi. The inocula were evenly spread out with the help of a sterile cotton swab. A standard disc diffusion method was used [10]. Sterile disc (Whatman's No.1; 6mm in diameter) were impregnated with 1ml: 1ml of the essential oils to DMSO and placed on the surface of the plate. The plates were incubated at 37^{0}C for 24 hours and zone of inhibition was measured and compared with the control. The tests were done in triplicates in each case. The magnitude of antimicrobial action was assessed by the diameter of inhibition zones (mm) and compared with the positive control antibiotic Ciprofloxacin, and Fluconazole as antifungal.

Determination of minimum inhibitory concentration (MIC).
The minimum inhibitory concentration (MIC) values were determined. The MIC is defined as the lowest concentration at which tested samples showed no visible bacterial growth after 24 hours incubation period at 37^{0}C. The essential oils were dissolved in DMSO, and then serial two-fold dilutions were made in a concentration range from 1ml: lml to 0.063ml/ml. The least concentration of the oil showing a clear zone of inhibition is meant to be taken as the MIC. However, the graphical method has been used here in order to get the actual MIC by extrapolation, using the line of best fit in the plot of inhibition zone diameter against the log of the concentration of the oil.

Statistical analysis.
All data are expressed as mean values ± standard deviation (S.D). Statistical analysis was performed using the SPSS software package (version 18.0). Differences on statistical analysis were considered significant at $p < 0.05$.

RESULTS

TABLE 1: Percent yield of Essential Oils extracted from the various plant materials.

Sample (fresh)	Weight (g)	Volume (ml)	Percentage yield (%v/w)
Ginger rhizome	50	1.8	3.60
African basil leaf	50	2.2	4.40
Onion bulb	50	1.5	3.00
Guava leaf	50	2.4	4.8

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IJFSNPHT (2017), 9(6):45-59

Table 2: Result of sensitivity test of extracted oils in DMSO (1ml: 1ml) against some selected pathogenic organisms (bacteria).

Treatment	Organism: MZID (mm)			
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
Ginger	12.5 ± 0.71	16.5 ± 0.71	14.5 ± 0.71	13.5 ± 0.71
African basil	9.5 ± 0.71	7.5 ± 0.71	27.5 ± 0.71	7.5 ± 0.71
Onion	7.5 ± 0.71	8.5 ± 0.71	7.5 ± 0.71	7.5 ± 0.71
Guava	11.5 ± 0.71	10.5 ± 0.71	8.5 ± 0.71	13.5 ± 0.71
Ciprofloxacin	19.5 ± 0.71	20.5 ± 0.71	13.5 ± 0.71	21.5 ± 0.71

The table above shows the antibacterial screening results of the various essential oils against the named organisms, recorded as Mean ± Standard Deviation (SD), of the zone of inhibition diameter (MZID). DMSO (negative control) showed no inhibition zone for all the bacteria, and fungi.

Table 3: Antifungal sensitivity test results.

Treatment	MZID (in mm)*
	Aspergillus niger
Ginger	9.5 ±0.71
African basil	10.5 ± 0.71
Onion	5.5 ± 0.71
Guava	7.5 ± 0.71
Fluconazole	15.5 ± 0.71

Key: * = Mean of Inhibition Zone Diameters

Table 4: Summary of MICs of Essential Oils against tested micro-organisms.

Essential Oil	MIC against Micro-organisms (ml/ml)				
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*
Ginger	0.05	0.001	0.008	0.126	0.006
African Basil	0.003	0.001	0.032	0.018	0.020
Onion	0.003	0.0003	0.004	0.016	0.063
Guava	0.032	0.025	0.002	0.112	0.079

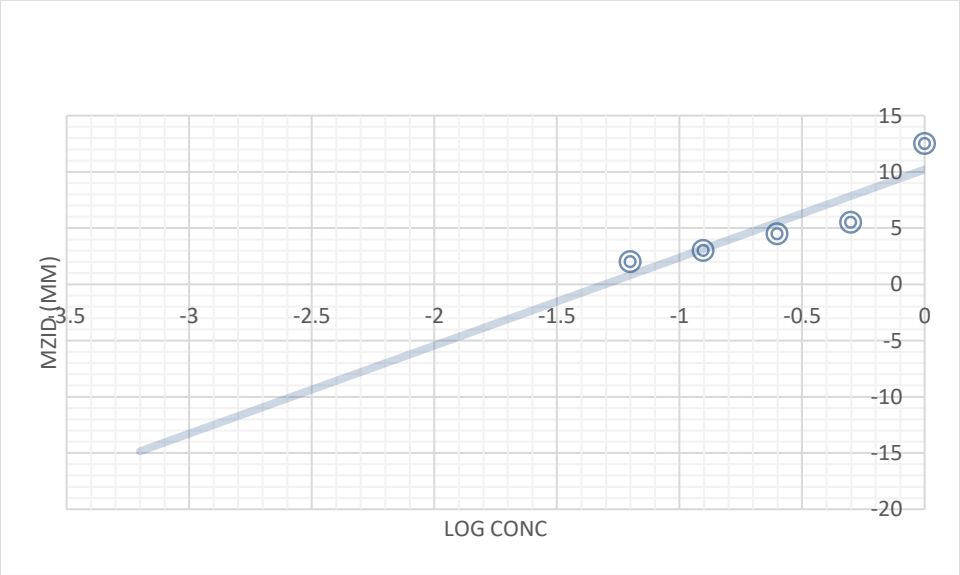

Fig. 1: Graph of MIC determination of Ginger oil on *Bacillus subtilis.*
MIC = antilog of intercept on x-axis (- 1.3) = 0.05

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

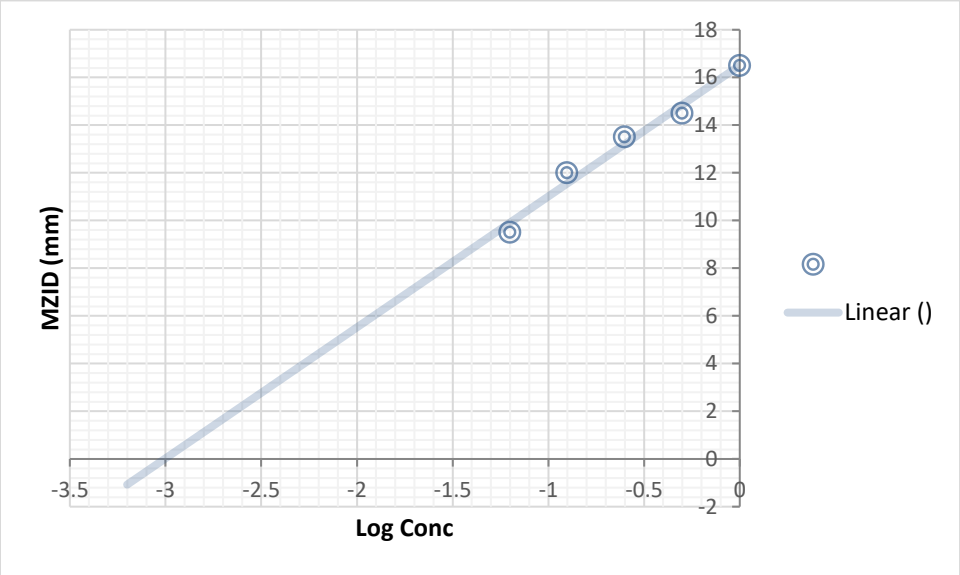

Fig. 2: Graph of MIC determination of Ginger oil on *Staphylococcus aureus.*
MIC = antilog of intercept on x-axis (-3.0) = 0.001

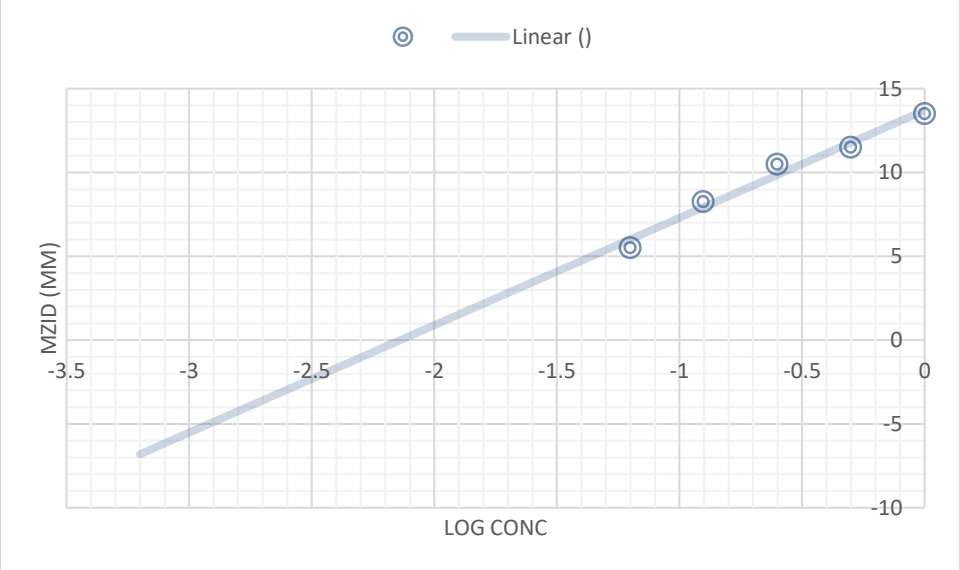

Fig. 3: Graph of MIC determination of Ginger oil on *Escherichia coli.*
MIC = antilog of intercept on x-axis (-2.1) = 0.008

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

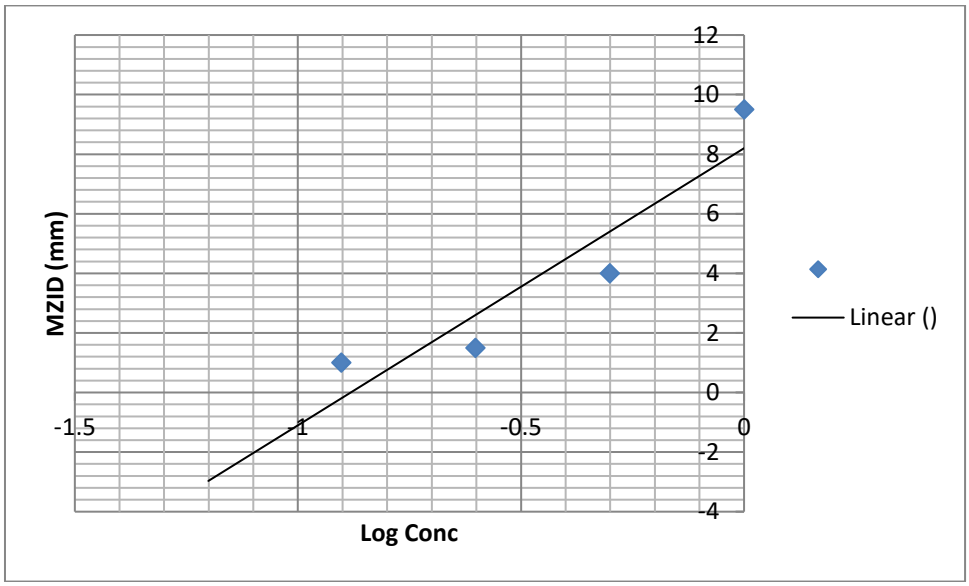

Fig. 4: Graph of MIC determination of Ginger oil on *Pseudomonas aeruginosa*
MIC = antilog of intercept on x-axis (-0.9) = 0.126

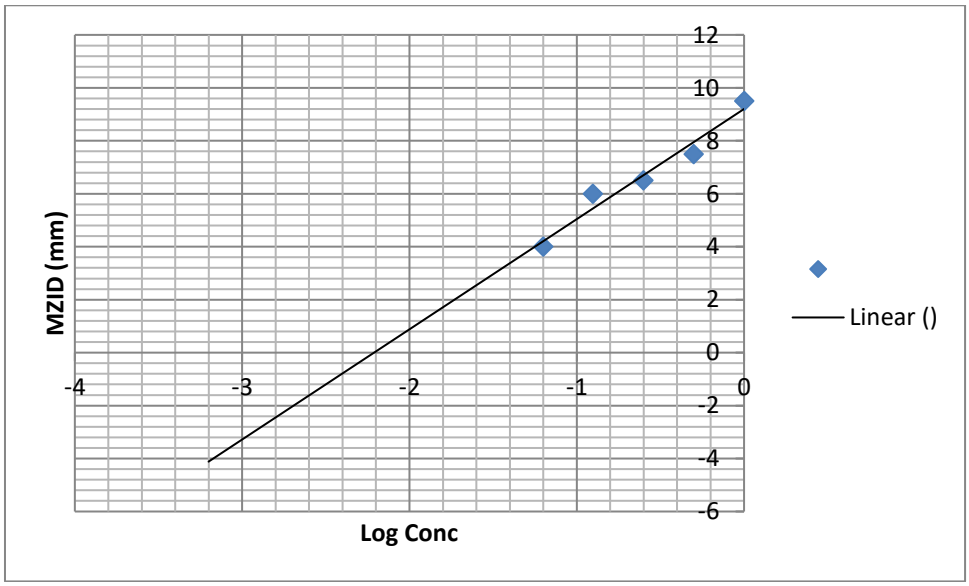

Fig. 5: Graph of MIC determination of Ginger oil on *Aspergillus niger.*
MIC = antilog of intercept on x-axis (-2.2) = 0.006

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

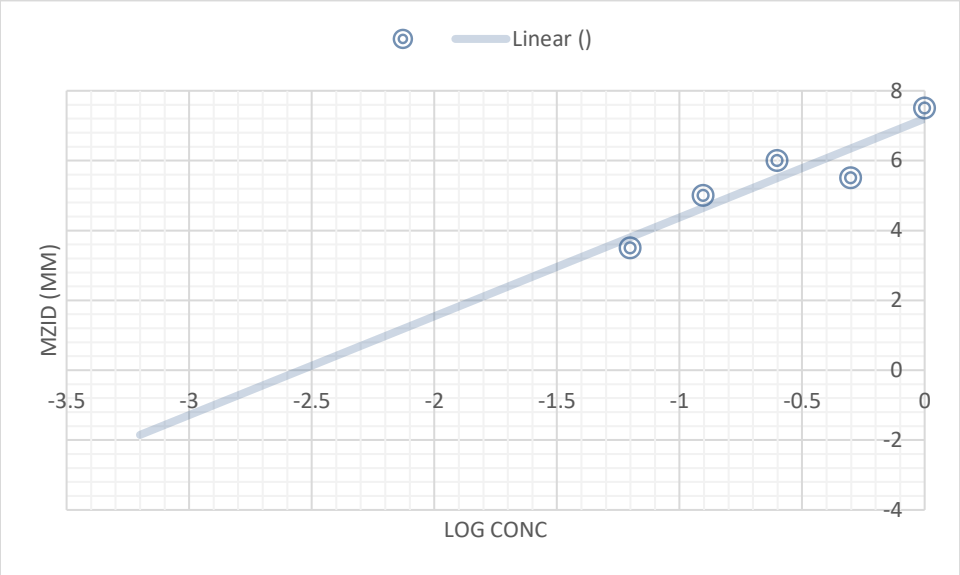

Fig. 6: Graph of MIC determination of African Basil oil on *Bacillus subtilis.* MIC = antilog of intercept on x-axis (-2.55) = 0.003

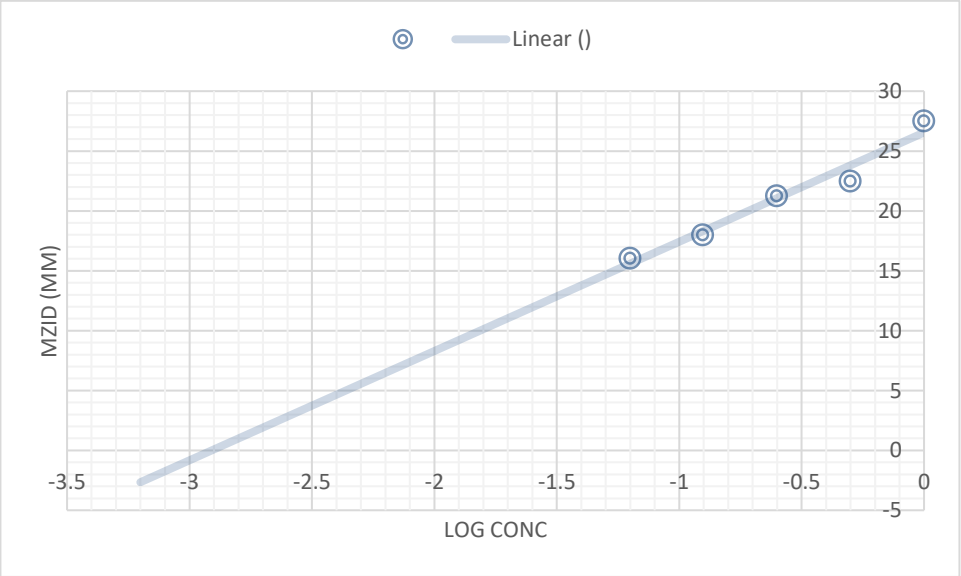

Fig. 7: Graph of MIC determination of African Basil oil on *Staphylococcus aureus.*
MIC = antilog of intercept on x-axis (-2.9) = 0.001

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

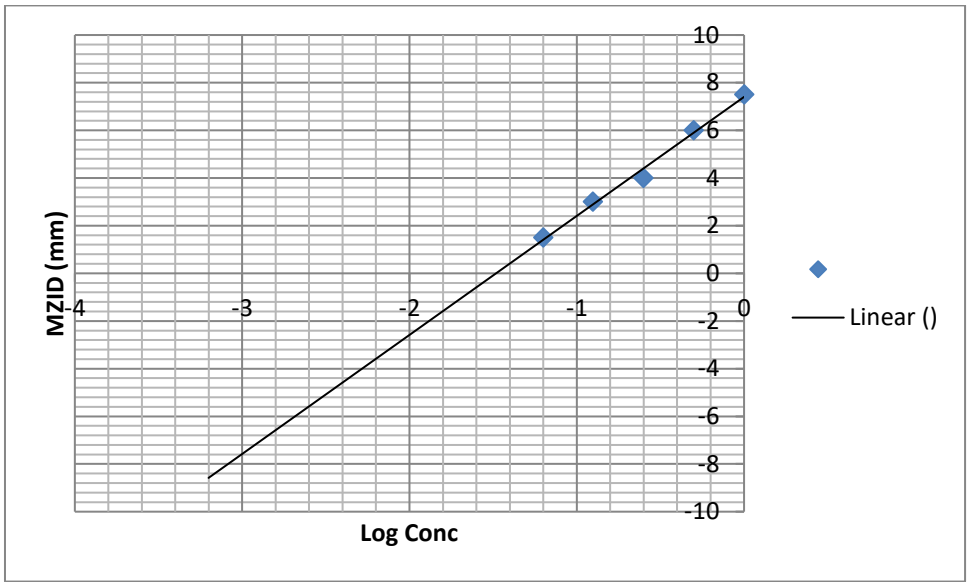

Fig. 8: Graph of MIC determination of African Basil oil on *Escherichia coli.*
MIC = antilog of intercept on x-axis (-1.5) = 0.032

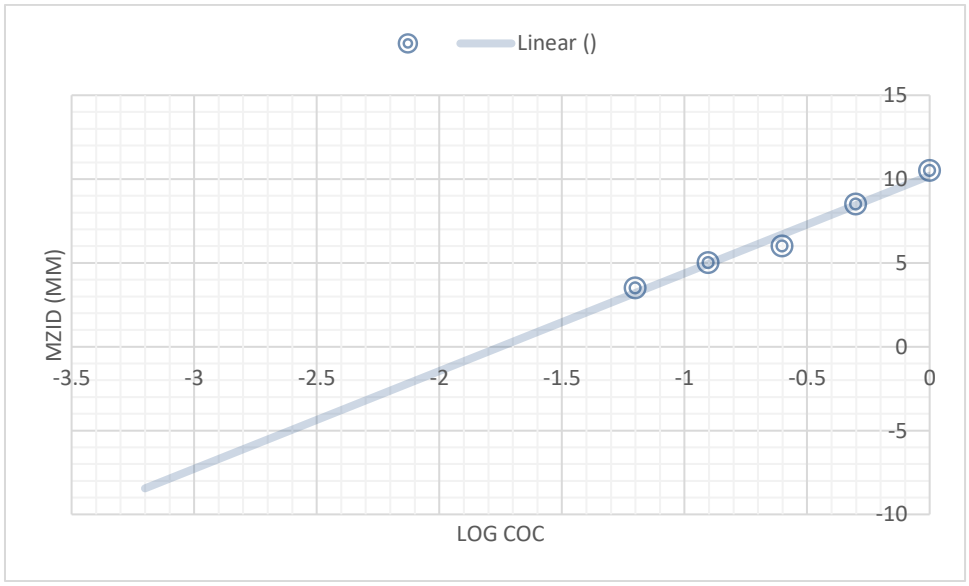

Fig. 9: Graph of MIC determination of African Basil oil on *Pseudomonas aeruginosa.*
MIC = antilog of intercept on x-axis (-1.75) = 0.018

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

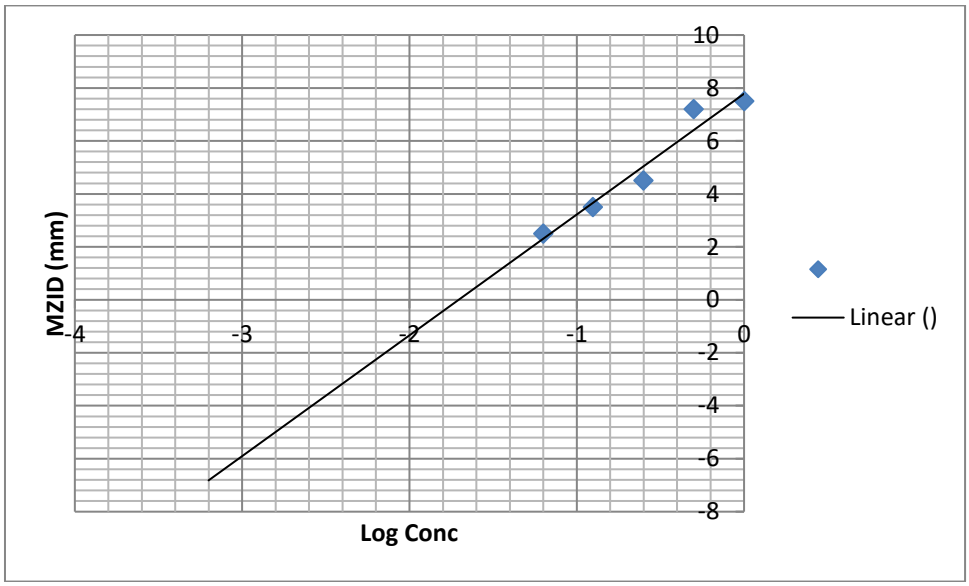

Fig. 10: Graph of MIC determination of African Basil oil on *Aspergillus niger.*
MIC = antilog of intercept on x-axis (-1.7) = 0.020

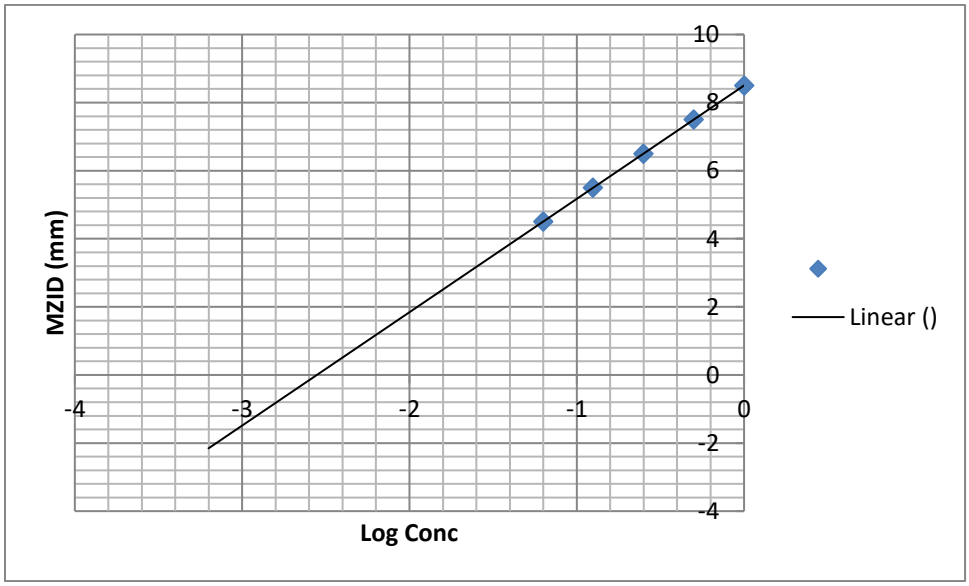

Fig. 11: Graph of MIC determination of Onion oil on *Bacillus subtilis.*
MIC = antilog of intercept on x-axis (-2.6) = 0.003

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

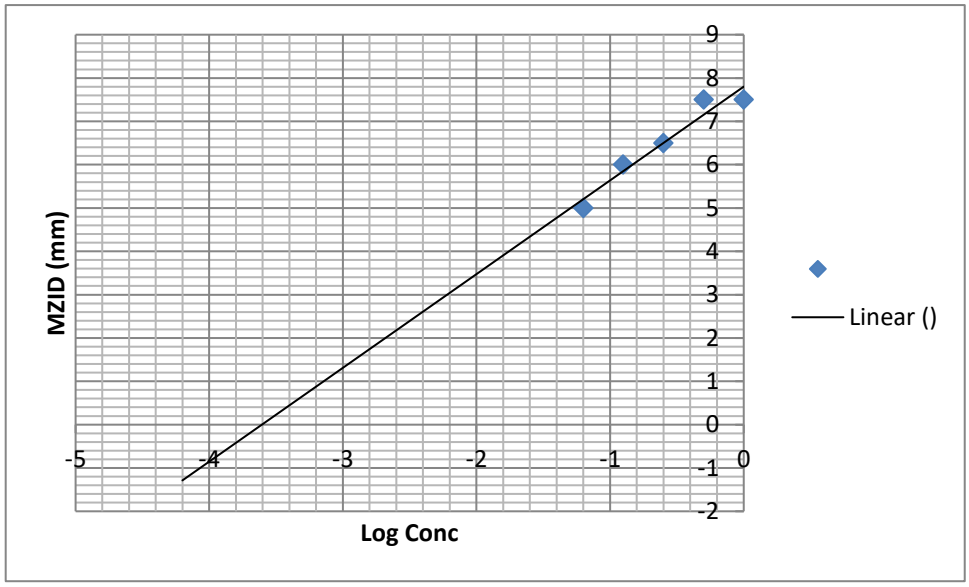

Fig. 12: Graph of MIC determination of Onion oil on *Staphylococcus aureus.*
MIC = antilog of intercept on x-axis (-3.6) = 0.0003

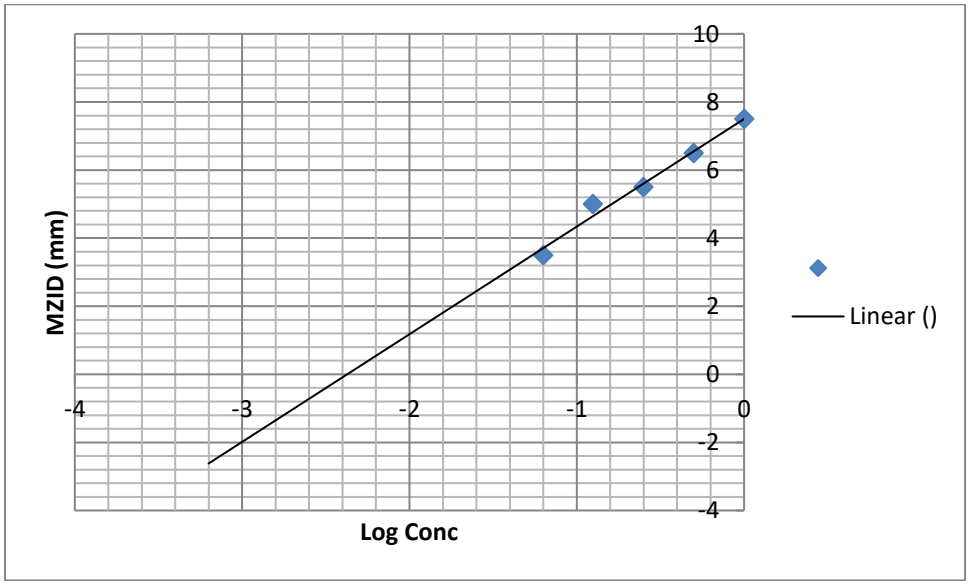

Fig. 13: Graph of MIC determination of Onion oil on *Escherichia coli.*
MIC = antilog of intercept on x-axis (-2.4) = 0.004

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

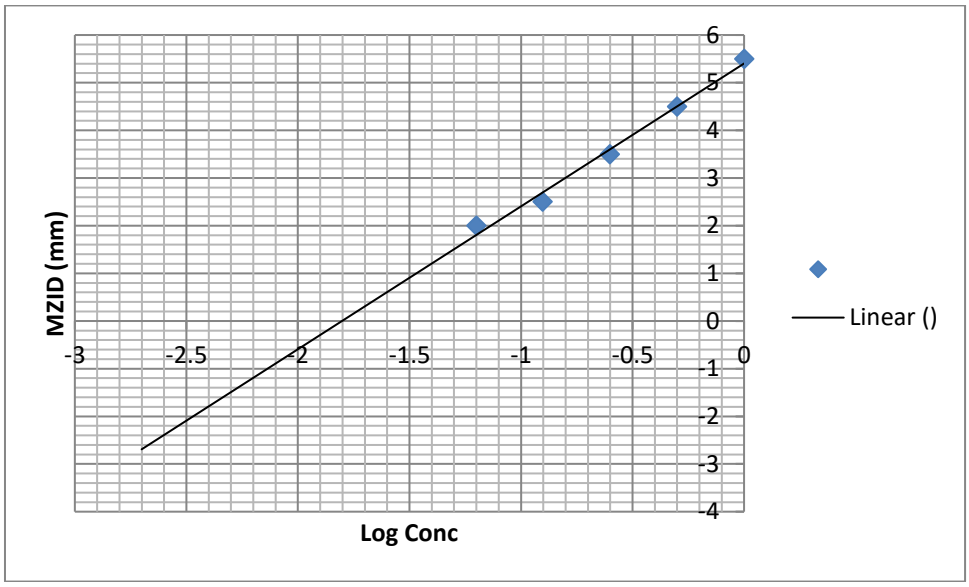

Fig. 14: Graph of MIC determination of Onion oil on *Pseudomonas aeruginosa.*
MIC = antilog of intercept on x-axis (-1.8) = 0.016

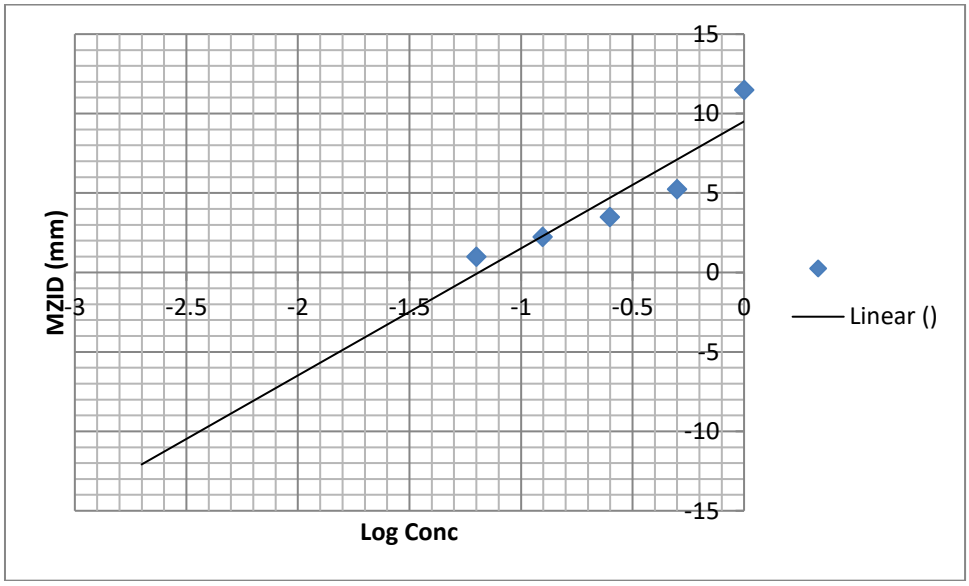

Fig. 15: Graph of MIC determination of Onion oil on *Aspergillus niger.*
MIC = antilog of intercept on x-axis (-1.2) = 0.063

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

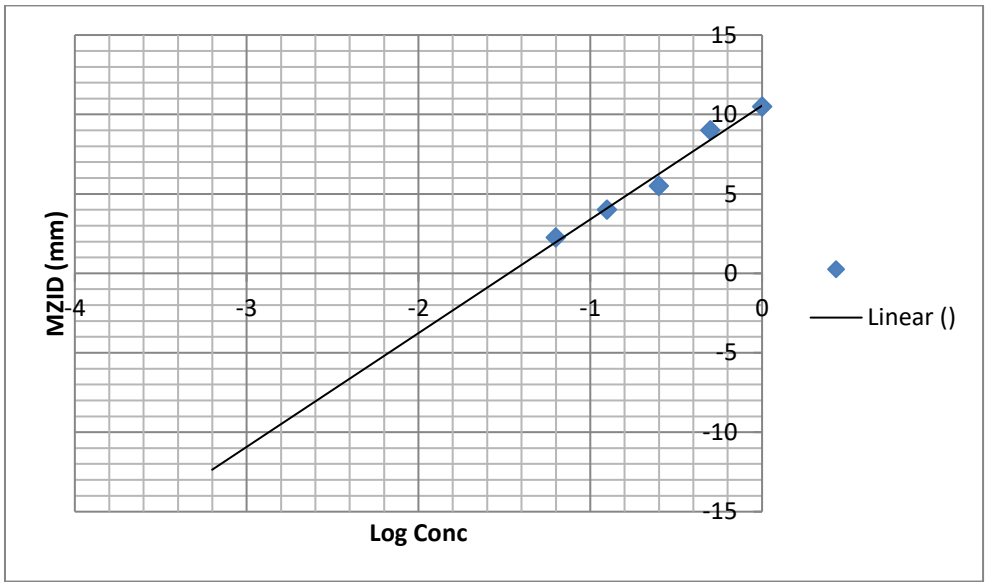

Fig. 16: Graph of MIC determination of Guava oil on *Bacillus subtilis.*
MIC = antilog of intercept on x-axis (-1.5) = 0.032

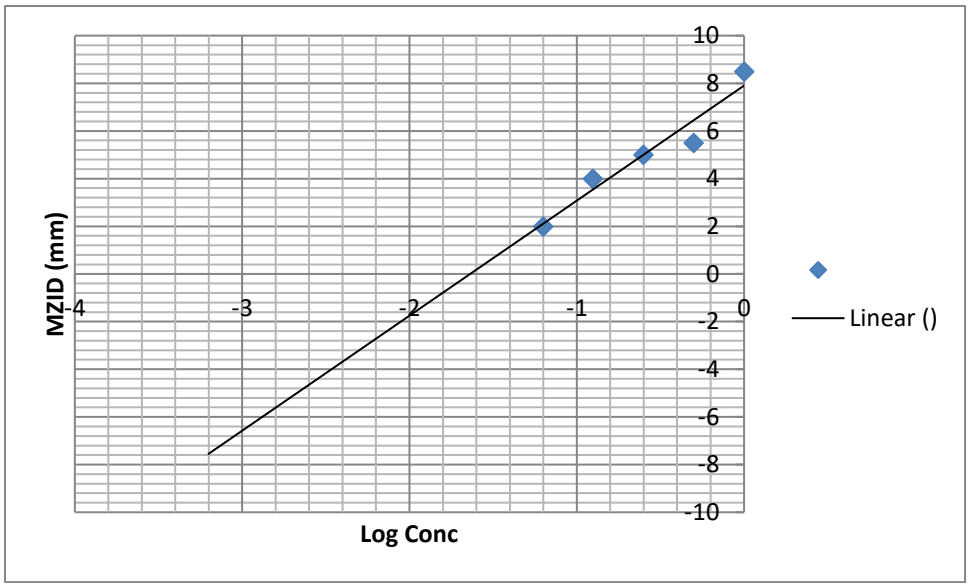

Fig. 17: Graph of MIC determination of Guava oil on *Staphylococcus aureus.*
MIC = antilog of intercept on x-axis (-1.6) = 0.025

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

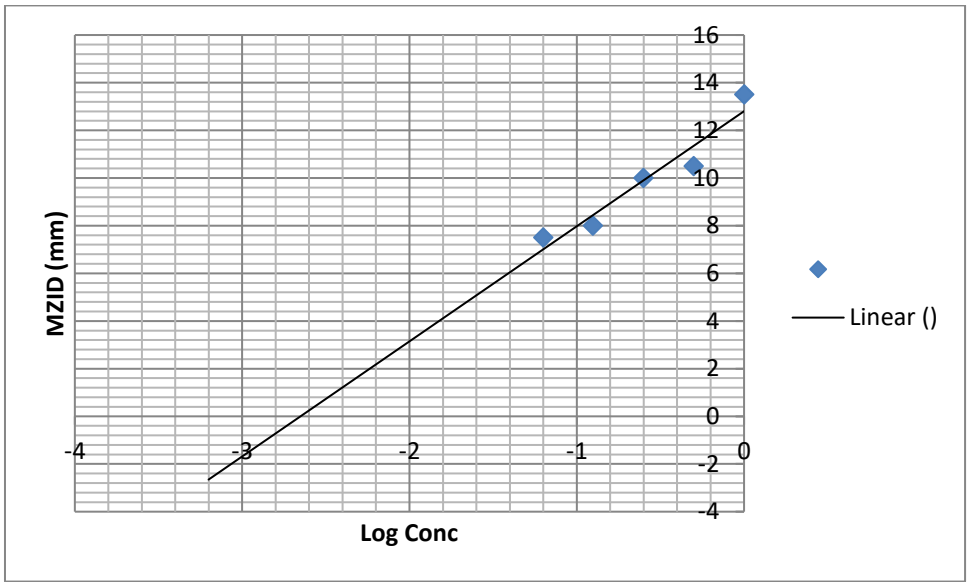

Fig. 18: Graph of MIC determination of Guava oil on *Escherichia coli.*
MIC = antilog of intercept on x-axis (-2.7) = 0.002

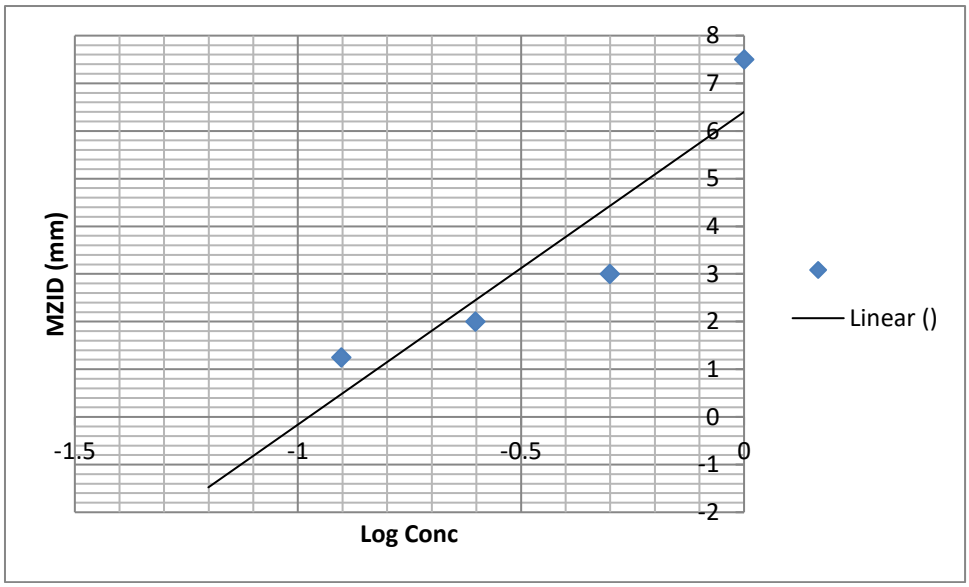

Fig. 19: Graph of MIC determination of Guava oil on *Pseudomonas aeruginosa.* MIC = antilog of intercept on x-axis (-0.95) = 0.112

ISSN: 0975 – 8712
IJFSNPHT (2017), 9(6):45-59

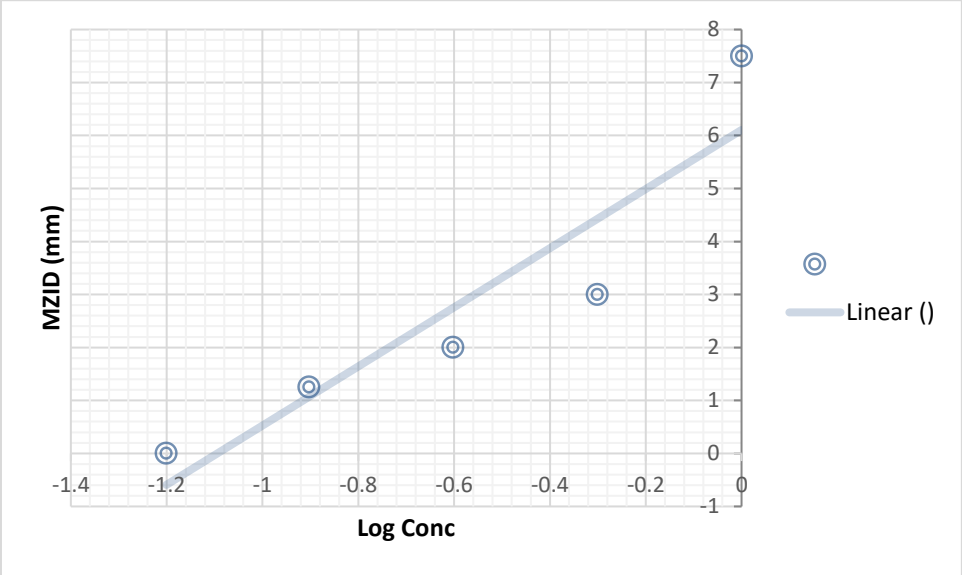

Fig. 20: Graph of determination of Guava oil on *Aspergillus niger.*
MIC = antilog of intercept on x-axis (-1.1) = 0.079

DISCUSSION

At a dose of 1ml of the oil, all the Essential Oils (EOs) tested caused inhibition of growth in all the bacteria and the fungi tested. The inhibition was found to be dose dependent leading to decreasing zones of inhibition as the doses decreased upon serial dilution, which agrees with the results reported by some workers [11]. Table.2, showing sensitivity results, indicates that the standard antibiotic used for this study as control, Ciprofloxacin, recorded the highest inhibition zone diameter than all the EOs, against all the bacteria tested, with the only exception being that of Basil against *E. coli* (27.5mm), which was significantly higher than that of Ciprofloxacin (13.5mm). This is in consonance with reports that Ciprofloxacin is an effective bactericidal agent against *P. aeruginosa* and *E. coli* [12]. Again, in the sensitivity test against the fungi, *Aspergillus niger*, the standard antifungal drug used as control, Fluconazole, showed the highest inhibition zone diameter of 15.5mm, compared to the EOs (Table.3). This is a pointer to the validity and reproducibility of the data in this study. The relative strengths of the essential oils against the micro-organisms was observed by comparing their Minimum Inhibition Concentrations (MICs), the smaller the MIC figure, the stronger the antimicrobial power of the EO against the micro-organisms [13].

Figures 1-5 show the graphical determination of the MIC of Ginger against the tested organisms, while Figures 6-10 are for that of Basil, Figures 11-15 for Onion, and Figures 16-20 for Guava. Table.4 shows a summary of the various MICs obtained from the graphs. The following deductions are made from Table.4. For the bacteria, *Bacillus subtilis* , *Ocimum gratissimum* (Basil) oil and *Allium cepa* (Onion) oil were found to be equally active in inhibiting its growth, and significantly ($p<0.05$) stronger than *Zingiber officinale* (Ginger) and *Psidium guajava* (Guava) oils. *B. subtilis* and *E.coli* have also been reported to be highly susceptible to the antibacterial activity of Fennel oil, an essential oil [14].

For *Staphylococcus aureus* , Ginger, Basil and Onion showed significantly ($p<0.05$) higher activity, with Guava being the weakest. In the case of *Escherichia coli*, the inhibitory power of the EOs was in the descending order Guava>Onion>Ginger, with Basil being the weakest. And for *Pseudomonas aeruginosa*, Onion and Basil were found to be significantly stronger than Guava and Ginger.

Against the fungi in this study, *Aspergillus niger*, Ginger showed a significantly higher activity followed by Basil, Onion and Guava. So, all these

EOs have been shown to be active against the fungi, *A. niger.* This is in agreement with previous reports [15], which stated that Ginger had the highest antifungal activity against all the tested fungi, including *A. niger*, followed by Garlic and Onion. In addition, it has been reported [16], that the ethanol extract of *O. gratissimum* (Basil) demonstrated a significant inhibitory activity against *A. niger*, while its chloroform extract has similar significant activity against same fungi [17]. Further, the essential oil from *O. gratissimum* has been shown to have antifungal activity against fungi such as *Candida albicans* [18].

Looking at the individual activities of the EOs (Table.4), Ginger was found to be active against the micro-organisms in the decreasing order *S.aureus>A.niger>E. coli>B. subtilis>P. aeruginosa*. The activity of Basil was in the order *S.aureus>B. subtilis>P.aeruginosa>A.niger>E. coli*; for Onion it was *S.aureus >B.subtilis >Ecoli >P.areuginosa >A.niger*; and for Guava it was *E.coli>S.aureus>B. subtilis>A. niger>P. aeruginosa.*

The results of these MIC determinations show that no single essential oil is consistently stronger than all the others against these particular bacteria or the fungi. The two exceptions are Onion with the lowest MIC (that is, highest activity) of 0.0003ml/ml against *S. aureus*, and Ginger with the highest MIC (that is, lowest activity) of 0.126ml/ml against *P. aeruginosa,* followed by Guava with an MIC of 0.112ml/ml against *P. aeruginosa.* On the other hand, *S.aureus* appears to be the most susceptible to all the essential oils with MICs as low as 0.001ml/ml for Ginger, 0.001ml/ml for Basil and 0.025ml/ml for Guava.
It is pertinent to note that *Staphylococcus aureus* which has been reported to show resistance to the first line drugs used to treat infections caused by it [19], has been found to be susceptible to all the EOs tested in this study.

CONCLUSION

All the EOs have demonstrated a broad spectrum of activity by being shown to be active against all the bacteria and fungi used in this study, however, none is consistently stronger against all the micro-organisms. That is, no specific pattern of inhibition has been observed; rather, what is apparent is that different EOs are active against the various micro-organisms to varying degrees. Therefore, any choice to be made as to which EO to be used as an antimicrobial for food preservation or therapy has to be made based on the specific sensitivity of the micro-organism to the particular EO. This study has also shown that the EOs from these medicinal plants harvested from the South East Nigeria have a similar antimicrobial profile as those reported by some workers elsewhere.

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